STIC-Biotech/Ch mLib 64605

Fr m: Hashemi, Shar
Sent: Tuesday, April 16, 2002 3:19 PM
To: STIC-Biotech/ChemLib
Subject: search request for 09/755398

Please search the following terms for application 09/755398 with filing date 1/4/01:
method
nucleic acid
analysis
DNA
RNA
deoxyribonucleic acid
quantitative expression analysis assay
QEA assay
poisoning
oligo-competition
extended oligo-competition
trace oligo-competition
extension
restriction endonuclease
primer

Point of Contact:
Mona Smith
Technical Information Specialist
CM1 6A01
Tel: 308-3278

References:
USP 5,202,231 (already have copy with appl)
USP 5,333,675

Abstract:
Disclosed are methods of selectively analyzing a nucleic acid in a sample. The methods allow for selective identification of a target sequence in a population of nucleic acids. For example, the methods allow for confirmation of the identity of a nucleic acid tentatively identified in a quantitative expression analysis assay.

Thank you.

Examiner Hashemi
703-305-4840
AU 1637
CM1 12D04

10E12

Searcher: M. Smith
Phone: _____
Location: _____
Date Picked Up: 4/20/02
Date Completed: 5/2/02
Searcher Prep/Review: 30
Clerical: _____
Online time: 35

TYPE OF SEARCH:
NA Sequences: _____
AA Sequences: _____
Structures: _____
Bibliographic: X
Litigation: _____
Full text: _____
Patent Family: _____
Other: _____

VENDOR/COST(where applic.)
STN: _____
DIALOG: _____
Questel/Orbit: _____
DRLink: _____
Lexis/Nexis: _____
Sequence Sys.: _____
WWW/Internet: _____
Other (specify): _____

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=> fil hcaplu
FILE 'HCAPLUS' ENTERED AT 11:15:59 ON 01 MAY 2002
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FILE COVERS 1907 - 1 May 2002  VOL 136 ISS 18
FILE LAST UPDATED: 29 Apr 2002  (20020429/ED)

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the CAS Roles thesaurus (/RL field) in this file.

=> d stat que
L1           34 SEA FILE=REGISTRY NUCLEIC ACID?/CN
L2        27471 SEA FILE=REGISTRY RIBONUCLEIC ACID?/CN
L3       253470 SEA FILE=REGISTRY DEOXYRIBONUCLEIC/BI
L4      1546735 SEA FILE=HCAPLUS L1 OR L2 OR L3 OR NUCLEIC(W)ACID? OR NA OR
                RIBONUCLEIC(W)ACID? OR RNA OR DEOXYRIBONUCLEIC(W)_ACID? OR DNA
L5            7 SEA FILE=HCAPLUS L4 AND (QEA OR QUANTITATIVE(W)EXPRESSION(W)ANA
                LYSIS)


=> d ibib abs hitrn 15 1-7

L5   ANSWER 1 OF 7  HCAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:         2001:507885  HCAPLUS
DOCUMENT NUMBER:          135:103339
TITLE:                    Method of identifying a candidate nucleic
                          acid sequence from a population of
                          nucleic acids using an improvement
                          of an oligo-competition QEA procedure
INVENTOR(S):              Bader, Joel S.; Gold, Steven; Gusev, Vladimir; Li, Shu
                          Xia; Shenoy, Suresh; Crasta, Oswald R.; Boufford,
                          Pascal
PATENT ASSIGNEE(S):       Curagen Corporation, USA
SOURCE:                   PCT Int. Appl., 42 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
```

LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001049886	A2	20010712	WO 2001-US300	20010105
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2002015951	A1	20020207	US 2001-755398	20010104
PRIORITY APPLN. INFO.:			US 2000-174685P P	20000106

AB Disclosed are methods of selectively analyzing a **nucleic acid** in a sample. The methods allow for selective identification of a target sequence in a population of **nucleic acids**. For example, the methods allow for confirmation of the identity of a **nucleic acid** tentatively identified in a quant. expression anal. assay. The **QEA** process involves a: fragmentation of cDNA pools with two different restriction enzymes, b: ligation of the restriction fragments to a FAM-labeled **DNA** adapter at one end of the fragment and a biotin-labeled **DNA** adapter at the second end of the fragment; c: PCR amplification of the ligated **DNA** mols. using primers specific to the sequences contained within the 2 adapter modules, which leads to the prodn. of approx. 300 fluorescent **DNA** fragments called **QEA** bands; d: purifn. of the biotin-labeled fragments on streptavidin-coated magnetic beads; and e: detn. of the size of the fragments by capillary electrophoresis of the purified of the purified **QEA** bands.

IT **9075-08-5**, Restriction endonuclease
RL: ARG (Analytical reagent use); BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(method of identifying a candidate **nucleic acid** sequence from a population of **nucleic acids** using an improvement of an oligo-competition **QEA** procedure)

L5 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:283960 HCAPLUS
DOCUMENT NUMBER: 132:318571
TITLE: Identification and comparison of protein-protein interactions and identification of inhibitors
INVENTOR(S): Nandabalan, Krishnan; Rothberg, Jonathan Marc; Yang, Meijia; Knight, James Robert; Kalbfleisch, Theodore Samuel
PATENT ASSIGNEE(S): Curagen Corporation, USA
SOURCE: U.S., 161 pp., Cont.-in-part of U.S. Ser. No. 663,824.
CODEN: USXXAM
DOCUMENT TYPE: Patent

LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6057101	A	20000502	US 1997-874825	19970613
US 6083693	A	20000704	US 1996-663824	19960614
CA 2257958	AA	19971218	CA 1997-2257958	19970613

PRIORITY APPLN. INFO.: US 1996-663824 A2 19960614

AB Methods are described for detecting protein-protein interactions, among two populations of proteins, each having a complexity of at least 1,000. For example, proteins are fused either to the **DNA**-binding domain of a transcriptional activator or to the activation domain of a transcriptional activator. Two yeast strains, of the opposite mating type and carrying one type each of the fusion proteins are mated together. Productive interactions between the two halves due to protein-protein interactions lead to the reconstitution of the transcriptional activator, which in turn leads to the activation of a reporter gene contg. a binding site for the **DNA**-binding domain. This anal. can be carried out for two or more populations of proteins. The differences in the genes encoding the proteins involved in the protein-protein interactions are characterized, thus leading to the identification of specific protein-protein interactions, and the genes encoding the interacting proteins, relevant to a particular tissue, stage or disease. Furthermore, inhibitors that interfere with these protein-protein interactions are identified by their ability to inactivate a reporter gene. The screening for such inhibitors can be in a multiplexed format where a set of inhibitors will be screened against a library of interactors. Further, information-processing methods and systems are described. These methods and systems provide for identification of the genes coding for detected interacting proteins, for assembling a unified database of protein-protein interaction data, and for processing this unified database to obtain protein interaction domain and protein pathway information. The method was used to select inhibitors of the interaction between R4 and FKBP-12.

REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:686628 HCAPLUS
DOCUMENT NUMBER: 131:318541
TITLE: Apparatus for identifying, classifying, or quantifying **DNA** sequences in a sample without sequencing
INVENTOR(S): Rothberg, Jonathan Marc; Deem, Michael W.; Simpson, John W.
PATENT ASSIGNEE(S): Curagen Corporation, USA
SOURCE: U.S., 110 pp., Cont.-in-part of U.S. 5,871,697. CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

US 5972693	A	19991026	US 1996-663823	19960614
US 5871697	A	19990216	US 1995-547214	19951024
WO 9715690	A1	19970501	WO 1996-US17159	19961024
W: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, FI, GE, HU, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9674763	A1	19970515	AU 1996-74763	19961024
AU 730830	B2	20010315		
EP 866877	A1	19980930	EP 1996-936985	19961024
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000500647	T2	20000125	JP 1997-516817	19961024
IL 124185	A1	20001206	IL 1996-124185	19961024
US 6141657	A	20001031	US 1997-942406	19971001
US 6231812	B1	20010515	US 1999-322617	19990528

PRIORITY APPLN. INFO.:

US 1995-547214	A2	19951024
US 1996-663823	A	19960614
WO 1996-US17159	W	19961024
US 1997-942406	A1	19971001

AB This invention provides methods, named Quant. Expression Anal. (**QEA**.RTM.), by which biol. derived **DNA** sequences in a mixed sample or in an arrayed single sequence clone can be detd. and classified without sequencing. The methods make use of information on the presence of carefully chosen target subsequences, typically of length from 4 to 8 base pairs, and preferably the length between target subsequences in a sample **DNA** sequence together with **DNA** sequence databases contg. lists of sequences likely to be present in the sample to det. a sample sequence. The preferred method uses restriction endonucleases to recognize target subsequences and cut the sample sequence. Then carefully chosen recognition moieties are ligated to the cut fragments, the fragments amplified, and the exptl. observation made. Polymerase chain reaction (PCR) is the preferred method of amplification. Several alternative embodiments are described which capable of increased discrimination and which use Type IIS restriction endonucleases, various capture moieties, or samples of specially synthesized cDNA. Another embodiment of the invention named colony calling (CC) uses information on the presence or absence of carefully chosen target subsequences in a single sequence clone together with **DNA** sequence databases to det. the clone sequence. Computer implemented methods are provided to analyze the exptl. results and to det. the sample sequences in question and to carefully choose target subsequences in order that expts. yield a max. amt. of information.

IT **9075-08-5**, Restriction endonuclease
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); CAT (Catalyst use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(method and programmable app. for detg. and classifying **nucleic acid** sequences in a sample without sequencing)

IT **9015-85-4**, **DNA** ligase

RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); CAT (Catalyst use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(method and programmable app. for detg. and classifying **nucleic acid** sequences in sample without sequencing)
REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:15865 HCAPLUS
DOCUMENT NUMBER: 128:71604
TITLE: Identification and comparison of protein-protein interactions that are tissue, development or disease specific and identification of inhibitors
INVENTOR(S): Nandabalan, Krishnan; Rothberg, Jonathan M.; Yang, Meijia; Knight, James R.; Kalbfleisch, Theodore S.
PATENT ASSIGNEE(S): Curagen Corporation, USA
SOURCE: PCT Int. Appl., 425 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9747763	A1	19971218	WO 1997-US10392	19970613
W: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GH, HU, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 6083693	A	20000704	US 1996-663824	19960614
CA 2257958	AA	19971218	CA 1997-2257958	19970613
AU 9733955	A1	19980107	AU 1997-33955	19970613
EP 912753	A1	19990506	EP 1997-930030	19970613
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: US 1996-663824 A 19960614
WO 1997-US10392 W 19970613

AB Disclosed are methods of detecting protein-protein interactions among two populations of proteins, wherein each protein population has a complexity of at least 1,000. Fusion proteins of each population are expressed in yeast cells of opposite mating types. The fusion protein populations are made by fusing to one population a **DNA**-binding domain of a transcriptional activator and fusing to the other population at the activation domain of a transcriptional activator. When the yeast cells of opposite mating type are mated, productive interactions between members of each protein population functionally reconstitute the two domains of the transcriptional activator and result in reporter gene expression. The disclosed methods allow identification and characterization of new protein-protein interactions that may be relevant to a particular tissue

or disease stage. Inhibitors of the identified protein-protein interactions can also be identified by screening for the ability to reverse expression of reporter gene. This inhibitor screening method can be performed in multiplex. Other aspects of the invention include information processing methods and systems. The methods and systems provide for assembling and processing of a unified database of sequences and identifying sequences that may be involved in protein-protein interactions. Exemplified was 5-fluoroorotic acid inhibition assay for selecting inhibitors of the interaction between R4 and FKBP-12.

L5 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:548721 HCAPLUS
DOCUMENT NUMBER: 115:148721
TITLE: NMR determination of order parameters in the quadrupolar glasses sodium cyanide chloride and sodium potassium cyanide
AUTHOR(S): Wiotte, W.; Petersson, J.; Blinc, R.; Elschner, S.
CORPORATE SOURCE: Fachbereich Phys., Univ. Saarlandes, Saarbruecken, D-6600, Fed. Rep. Ger.
SOURCE: Phys. Rev. B: Condens. Matter (1991), 43(16-A), 12751-66
CODEN: PRBMDO; ISSN: 0163-1829
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Quadrupolar perturbed NMR is a powerful method to investigate the quadrupolar glasses **Na**(CN)xCl1-x and NaxK1-xCN. In both systems at the **Na** and Cl sites, distributions of elec.-field-gradient tensors occur which are restricted by the fact that the av. structure of the systems under investigation is cubic. Correspondingly, inhomogeneous distributions of NMR lines result, which for I = 3/2 nuclear-spin systems consist of inhomogeneously broadened central lines and broad distributions of satellite lines. Measurements of these frequency distributions and their dependences on the compn., the orientation, and the temp. of the samples are presented. The widths of the elec.-field-gradient-tensor distributions are related in a general quadrupolar glass model to the quadrupolar Edwards-Anderson order parameter **qEA**. As a consequence, the temp. dependence of **qEA** is derived, reflecting the random orientational freeze-out of the CN quadrupoles with decreasing temp. By interpreting the results in terms of theor. models, it is shown that in the mixed cyanides one deals with a smearing of a collective quadrupolar glass transition by weak random fields and not with a pure random-field-type freezing or a pure random-bond-type glass transition. The crit. elastic behavior of these systems is discussed in a general context.

L5 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1977:23657 HCAPLUS
DOCUMENT NUMBER: 86:23657
TITLE: Cut-off criteria of electronic partition functions: effects on spectroscopic quantities
AUTHOR(S): Capitelli, M.; Ferraro, G.
CORPORATE SOURCE: Cent. Stud. Chim. Plasmi, Univ. Bari, Bari, Italy
SOURCE: Spectrochim. Acta, Part B (1976), 31B(5), 323-6
CODEN: SAASBH

DOCUMENT TYPE: Journal
LANGUAGE: English
AB The ratio of the no. d. of atoms or ions (**na**) to its electronic partition function (**Qea**) required to det. the abs. intensity of a spectral line in a plasma, was calcd. by 4 methods used for calg. local thermodn. equil. properties of plasmas. Semiempirical methods, such as the ground-state or few-level methods, are sufficiently accurate and **na/Qea** is independent of cut-off criteria.

L5 ANSWER 7 OF 7 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1975:417419 HCAPLUS
DOCUMENT NUMBER: 83:17419
TITLE: Possibility of obtaining a plasma of a working agent in the channel of a magnetohydrodynamic generator with irradiation by .alpha.-particles
AUTHOR(S): Kuznetsova, E. F.; Posvstugar, V. I.; Radchenko, R. V.
CORPORATE SOURCE: USSR
SOURCE: Teplofiz. Termodin. (1974), 48-53. Editor(s): Skripov, V. P.; Sheinkman, A. G. Akad. Nauk SSSR, Ural. Nauchn. Tsentr: Sverdlovsk, USSR. CODEN: 30EKAR
DOCUMENT TYPE: Conference
LANGUAGE: Russian
AB One of the most important methods for nonthermal ionization of a plasma is irradn. by .alpha.-particles in a MHD generator channel. The possibility was therefore examd. of obtaining an .alpha.-emitting isotope by the (n,.gamma.) reaction in a reactor-generator. The efficiency of MHD generator in combination with such a reactor is evaluated. Theor. equations involving the thermal and net power, Wr and Wp, resp., are given and the relation Wr/Wp for He [7440-59-7] as the working substance of a MHD generator (with 209Bi as the element undergoing n bombardment in an assocd. reactor-generator fueled with 235U) was computed. The elastic scattering cross section of e (in this case, resulting from .alpha.-particle interaction with the MHD generator walls) on He atoms is 5 .times. 10-16 cm2 and is practically const. in the temp. range (1000-2000.degree.K) studied. The contribution from thermal ionization at 800-2000.degree.K was negligibly small. Calcd. values of the ratio Wr/Wp are tabulated. Wr/Wp depends strongly on the product naQea (where **na** is the at. d. and **Qea** is the elastic scattering cross section of e on atoms; it is min. with Ar [7440-37-1] as a working substance. Helium cannot be recommended as a working substance for MHD generators with ionization by .alpha.-particles. From an economical viewpoint, ionization by .alpha.-particles cannot compete with the usual method of thermal ionization.

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? show files
File 155:MEDLINE(R) 1966-2002/Apr W4
File   5:Biosis Previews(R) 1969-2002/Apr W4
         (c) 2002 BIOSIS
File  10:AGRICOLA 70-2002/Apr
         (c) format only 2002 The Dialog Corporation
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         (c) 2002 Inst for Sci Info
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         (c) 2002 ProQuest Info&Learning
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         (c) 2002 Cambridge Sci Abs
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         (c) 2002 INIST/CNRS
File 351:Derwent WPI 1963-2001/UD,UM &UP=200227
         (c) 2002  Thomson Derwent
File 357:Derwent Biotech Res 1982-2002/Feb w3
         (c) 2002 Thomson Derwent & ISI
File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
         (c) 1998 Inst for Sci Info
File 440:Current Contents Search(R) 1990-2002/May 02
         (c) 2002 Inst for Sci Info
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Set     Items   Description
S1         54   (NA OR DNA OR RNA OR (NUCLEIC OR RIBONUCLEIC OR DEOXYRIBON-
               UCLEIC)(W)ACID?) AND (QEA OR QUANTITATIVE(W)EXPRESSION(W)ANAL-
               YSIS)
S2         28   RD (unique items)
? t1/3 ab/1-28
>>>No matching display code(s) found in file(s): 65

 1/AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12527534   21376466   PMID: 11483218
   Comparison of RNA and cDNA transfection methods for rescue of
infectious bursal disease virus.
  Boot H J; Dokic K; Peeters B P
  Department of Avian Virology, Institute for Animal Science and Health,
ID-Lelystad, PO Box 65, NL-8200 AB, Lelystad, The Netherlands.
h.j.boot@id.wag-ur.nl
  Journal of virological methods (Netherlands) Sep 2001, 97 (1-2)
p67-76, ISSN 0166-0934 Journal Code: 8005839
  Languages: ENGLISH
  Document type: Journal Article
```

Record type: In Process
Specific alterations in the genetic material of **RNA** viruses rely on a technique known as reverse genetics. Transfection of cells with the altered generic material is a critical step of this procedure. In this report we have compared **RNA** and cDNA transfection methods for the efficiency of transient protein expression and rescue of (recombinant) infectious bursal disease virus (IBDV). **Quantitative expression analysis** of the secreted alkaline phosphatase reporter protein, and qualitative expression levels of an IBDV protein showed both that cDNA transfection results in a much higher level of protein expression than **RNA** transfection. Because the rescue of a crippled variant of IBDV was achieved consistently using the cDNA transfection method, but failed when we used the **RNA** transfection method, we favor the cDNA transfection method for the rescue of (recombinant) IBDV from cloned cDNA.

1/AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11657902 21275464 PMID: 11381030
Comprehensive genome sequence analysis of a breast cancer amplicon.
Collins C; Volik S; Kowbel D; Ginzinger D; Ylstra B; Cloutier T; Hawkins T; Predki P; Martin C; Wernick M; Kuo WL; Alberts A; Gray JW
University of California San Francisco Cancer Center, San Francisco, California 94143-0808, USA. collins@cc.ucsf.edu
Genome research (United States) Jun 2001, 11 (6) p1034-42, ISSN 1088-9051 Journal Code: CES
Contract/Grant No.: CA 58207, CA, NCI
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Gene amplification occurs in most solid tumors and is associated with poor prognosis. Amplification of 20q13.2 is common to several tumor types including breast cancer. The 1 Mb of sequence spanning the 20q13.2 breast cancer amplicon is one of the most exhaustively studied segments of the human genome. These studies have included amplicon mapping by comparative genomic hybridization (CGH), fluorescent in-situ hybridization (FISH), array-CGH, quantitative microsatellite analysis (QUMA), and functional genomic studies. Together these studies revealed a complex amplicon structure suggesting the presence of at least two driver genes in some tumors. One of these, ZNF217, is capable of immortalizing human mammary epithelial cells (HMEC) when overexpressed. In addition, we now report the sequencing of this region in human and mouse, and on quantitative expression studies in tumors. Amplicon localization now is straightforward and the availability of human and mouse genomic sequence facilitates their functional analysis. However, comprehensive annotation of megabase-scale regions requires integration of vast amounts of information. We present a system for integrative analysis and demonstrate its utility on 1.2 Mb of sequence spanning the 20q13.2 breast cancer amplicon and 865 kb of syntenic murine sequence. We integrate tumor genome copy number measurements with exhaustive genome landscape mapping, showing that amplicon boundaries are associated with maxima in repetitive element density and a region of evolutionary instability. This integration of comprehensive sequence annotation, **quantitative expression analysis**, and tumor

amplicon boundaries provide evidence for an additional driver gene prefoldin 4 (PFDN4), coregulated genes, conserved noncoding regions, and associate repetitive elements with regions of genomic instability at this locus.

1/AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10511240 20163624 PMID: 10701689
Methacarn fixation: a novel tool for analysis of gene expressions in paraffin-embedded tissue specimens.
Shibutani M; Uneyama C; Miyazaki K; Toyoda K; Hirose M
Division of Pathology, National Institute of Health Sciences, Kamiyoga, Tokyo, Japan. shibutan@nihs.go.jp
Laboratory investigation (UNITED STATES) Feb 2000, 80 (2) p199-208, ISSN 0023-6837 Journal Code: KZ4
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
To establish a quantitative method for analysis of gene expressions in small areas of tissue after paraffin embedding, preliminary validation experiments with RT-PCR and Western blotting were performed using methacarn-fixed rodent tissues and a cultured PC12 cell line. A total **RNA** yield of 52 +/- 15 ng/mm2, sufficient for a quantitative RT-PCR of many genes, could be extracted from a deparaffinized 10-microm-thick rat-liver section by a simple, single-step extraction method. The low concentration of contaminating genomic **DNA** and the resolution of ribosomal RNAs in **RNA** gel proved the purity and integrity of the extracted **RNA** samples, allowing PCR amplification of a long mRNA sequence and mRNA species expressing low copy numbers. PCR amplification of mRNA-derived target gene fragments could be achieved by optimizing the amount of total **RNA** for reverse transcription and the number of subsequent PCR cycles for each gene. By this validation, organ- and sex-specific mRNA expression could be detected in methacarn-fixed paraffin-embedded tissues without additional DNase treatment of **RNA** samples. RT-PCR analysis could also be performed with total **RNA** extracted from deparaffinized tissue dissected with a laser capture microdissection system. In addition, extraction of protein yielded 4.9 +/- 2.1 microg/mm2 from a 10-microm-thick rat-liver section, allowing a **quantitative expression analysis** of protein by Western blotting. Thus, in addition to its advantages for immunohistochemistry, methacarn-fixed paraffin-embedded tissue has benefits for analysis of both RNAs and proteins in the cells of histologically defined areas.

1/AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10327880 99122773 PMID: 9925364
Distinct molecular phenotypes in murine cardiac muscle development, growth, and hypertrophy.
Schoenfeld JR; Vasser M; Jhurani P; Ng P; Hunter JJ; Ross J; Chien KR; Lowe DG

Department of Cardiovascular Research, Genentech, Inc., South San Francisco, CA 94080, USA.
Journal of molecular and cellular cardiology (ENGLAND) Nov 1998, 30 (11) p2269-80, ISSN 0022-2828 Journal Code: J72
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The onset of cardiac hypertrophy is associated with characteristic changes in myocardial gene expression that are thought to recapitulate a developmental gene program. We report here the first gene expression profile of the murine myocardium, using a rapid method of **quantitative expression analysis** based on real-time analytical RT-PCR. This assay was used to measure expression levels of 29 genes in (1) late stage development as represented by day 1 neonatal ventricles, (2) normal cardiac growth in 3 and 18 month old mice, and (3) cardiac hypertrophy following pressure overload by aortic constriction. For males and females normal growth is not associated with differential expression although there is elevated expression of skeletal and smooth muscle actin mRNA's in males compared to females. Using normal adult ventricles as a reference, there are many qualitative and quantitative differences between the day 1 neonatal myocardium and experimental cardiac hypertrophy. These data suggest that the response to POL involves a subset of re-expressed developmental genes together with altered expression of genes not necessarily associated with cardiac development.

1/AB/5 (Item 1 from file: 5)

13545029 BIOSIS NO.: 200200173850
Quantitative expression analysis of the cellular specificity of HECT-domain ubiquitin E3 ligases.
AUTHOR: Scarafia Liliana E; Winter Andreas; Swinney David C(a)
AUTHOR ADDRESS: (a)Roche Bioscience, 3401 Hillview Ave., Palo Alto, CA, 94304**USA E-Mail: david.swinney@roche.com
JOURNAL: Physiological Genomics 4p147-153 February, 2001
MEDIUM: print
ISSN: 1094-8341
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We evaluated the expression of 28 gene sequences with homology to the carboxy terminal of HECT E3 ubiquitin ligases in nine human cell lines using RT-PCR, to determine whether gene expression could be associated with cell-specific functions (HECT is "homologous to E6AP C-terminus"). In general, HECT-domain E3 ligases are constitutively expressed at low levels with a broad range between cell types. hetch3, 21, and 23 had higher levels in three leukocytic lines (Jurkat, MM6, THP1); hecth11 was more abundant in HepG2 and A495; and hecth15 and hecth12 were differentially expressed in lung fibroblasts derived from normal and severe emphysema patients (CCD16 and CCD29, respectively). Absolute quantitation showed that most HECT E3s have about 20-100 copies

of mRNA per Jurkat cell. By comparison, UBCH7 (an ubiquitin-conjugating E2) is 10-fold more abundant in Jurkat cells and 30-fold more abundant than E2 UBCH5A. We interpret the broad range of transcript levels to be consistent with the hypothesis that the concentrations of E3 are important for ubiquitination selectivity, leading us to conclude that substrate activation is necessary but not sufficient for selectivity.

2001

1/AB/6 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13260125 BIOSIS NO.: 200100467274
Comparison of **RNA** and cDNA transfection methods for rescue of infectious bursal disease virus.
AUTHOR: Boot Hein J(a); Dokic Kristina; Peeters Ben P H
AUTHOR ADDRESS: (a)Department of Avian Virology, Institute for Animal Science and Health, ID-Lelystad, NL-8200 AB, Lelystad: h.j.boot@id.wag-ur.nl**Netherlands
JOURNAL: Journal of Virological Methods 97 (1-2):p67-76 September, 2001
MEDIUM: print
ISSN: 0166-0934
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Specific alterations in the genetic material of **RNA** viruses rely on a technique known as reverse genetics. Transfection of cells with the altered generic material is a critical step of this procedure. In this report we have compared **RNA** and cDNA transfection methods for the efficiency of transient protein expression and rescue of (recombinant) infectious bursal disease virus (IBDV). **Quantitative expression analysis** of the secreted alkaline phosphatase reporter protein, and qualitative expression levels of an IBDV protein showed both that cDNA transfection results in a much higher level of protein expression than **RNA** transfection. Because the rescue of a crippled variant of IBDV was achieved consistently using the cDNA transfection method, but failed when we used the **RNA** transfection method, we favor the cDNA transfection method for the rescue of (recombinant) IBDV from cloned cDNA.

2001

1/AB/7 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12593156 BIOSIS NO.: 200000346658
Quantitative expression analysis of genes regulated by both obesity and leptin reveals a regulatory loop between leptin and

pituitary-derived ACTH.
AUTHOR: Renz Mark; Tomlinson Elizabeth; Hultgren Bruce; Levin Nancy; Gu Qimin; Shimkets Richard A; Lewin David A; Stewart Timothy A(a)
AUTHOR ADDRESS: (a)Dept. of Endocrine Research, Genentech, Inc., 1 DNA Way, South San Francisco, CA, 94080**USA
JOURNAL: Journal of Biological Chemistry 275 (14):p10429-10436 April 7, 2000
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Absence of the hormone leptin leads to dramatic increases in appetite, food intake, and adiposity. The primary site of action, at least with respect to appetite, is the hypothalamus. Leptin also has significant effects on the function(s) of peripheral organs involved in maintaining body composition. Some of these effects are mediated through direct interaction of leptin with its receptor on the target tissue, and some effects are indirectly mediated through secondary hormonal and neural pathways. Few of the genes that are responsible for regulating body composition and the peripheral effects of leptin are known. We have used a new gene profiling technology to characterize gene expression changes that occur in the pituitary, hypothalamus, fat, muscle, and liver in response to both obesity and treatment with exogenous leptin. These differences were then overlaid to allow the identification of genes that are regulated by obesity and at least partially normalized by leptin treatment. By using this process we have identified five genes (POMC, PC2, prolactin, HSGP25L2G, and one novel) that are both abnormally expressed in the pituitaries of obese mice and are sensitive to the effects of leptin. We also show that adreno-corticotropic hormone appears to be involved in a regulatory loop involving leptin.

2000

1/AB/8 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12522247 BIOSIS NO.: 200000275749
Identification of vitamin D 24 hydroxylase (CYP24) as a candidate oncogene by microarray CGH and **quantitative expression analysis.**

AUTHOR: Ylstra Bauke(a); Livezey Kristin W(a); Albertson Donna G(a)
AUTHOR ADDRESS: (a)UCSF Cancer Ctr, San Francisco, CA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting (41):p859 March, 2000
MEDIUM: print.
CONFERENCE/MEETING: 91st Annual Meeting of the American Association for Cancer Research. San Francisco, California, USA April 01-05, 2000
ISSN: 0197-016X
RECORD TYPE: Citation

LANGUAGE: English
SUMMARY LANGUAGE: English
2000

1/AB/9 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12431156 BIOSIS NO.: 200000184658
Methacarn fixation: A novel tool for analysis of gene expressions in paraffin-embedded tissue specimens.
AUTHOR: Shibutani Makoto(a); Uneyama Chikako; Miyazaki Keiko; Toyoda Kazuhiro; Hirose Masao
AUTHOR ADDRESS: (a)Division of Pathology, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo, 158-8501**Japan
JOURNAL: Laboratory Investigation 80 (2):p199-208 Feb., 2000
ISSN: 0023-6837
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: To establish a quantitative method for analysis of gene expressions in small areas of tissue after paraffin embedding, preliminary validation experiments with RT-PCR and Western blotting were performed using methacarn-fixed rodent tissues and a cultured PC12 cell line. A total **RNA** yield of 52 +- 15 ng/mm2, sufficient for a quantitative RT-PCR of many genes, could be extracted from a deparaffinized 10-mum-thick rat-liver section by a simple, single-step extraction method. The low concentration of contaminating genomic **DNA** and the resolution of ribosomal RNAs in **RNA** gel proved the purity and integrity of the extracted **RNA** samples, allowing PCR amplification of a long mRNA sequence and mRNA species expressing low copy numbers. PCR amplification of mRNA-derived target gene fragments could be achieved by optimizing the amount of total **RNA** for reverse transcription and the number of subsequent PCR cycles for each gene. By this validation, organ- and sex-specific mRNA expression could be detected in methacarn-fixed paraffin-embedded tissues without additional DNase treatment of **RNA** samples. RT-PCR analysis could also be performed with total **RNA** extracted from deparaffinized tissue dissected with a laser capture microdissection system. In addition, extraction of protein yielded 4.9 +- 2.1 mug/mm2 from a 10-mum-thick rat-liver section, allowing a **quantitative expression analysis** of protein by Western blotting. Thus, in addition to its advantages for immunohistochemistry, methacarn-fixed paraffin-embedded tissue has benefits for analysis of both RNAs and proteins in the cells of histologically defined areas.

2000

1/AB/10 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

04632715 BIOSIS NO.: 000079045752
FAILURE OF CHANGES IN INTRACAPILLARY PRESSURES TO ALTER PROXIMAL FLUID REABSORPTION
AUTHOR: BANK N; AYNEDJIAN H S
AUTHOR ADDRESS: RENAL ELECTROLYTE AND HYPERTENSION DIV., MONTEFIORE MED. CENTER, 111 EASTR 210TH ST., BRONX, NEW YORK 10467, USA.
JOURNAL: KIDNEY INT 26 (3). 1984. 275-282. 1984
FULL JOURNAL NAME: Kidney International
CODEN: KDYIA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: To determine the role that peritubular capillary oncotic and hydraulic pressures play in regulating urinary **Na** excretion (UNaV) in the euvolemic state, experiments were carried out in rats under conditions which altered these pressures without volume expanding the animal. In cross-circulation experiments, the donor rat was expanded with plasma or Ringer's solution while the recipient rat remained euvolemic. Micropuncture measurements in the euvolemic recipients demonstrated significant increases in efferent plasma flow rate (**QEA**), capillary hydraulic pressure (Pc) and decreases in mean capillary oncotic pressure (.hivin..pi.c). There were no changes in single nhephron glomerular filtration rate (SNGFR), absolute proximal reabsorption (APR), or UNa V. In additional studies, peritubular oncotic pressure was lowered markedly by plasmapheresis of the experimental animal. Large decreases in .hivin..pi.c were produced without any change occurring in SNGFR, APR, or UNa V. Measurements of interstitial hydraulic pressure (Pi) with a subcapsular pressure pipet revealed that Pi was unaltered under all of these conditions but rose markedly in rats undergoing a saline-expansion diuresis. APR and UNa V can remain constant despite large changes in .hivin..pi.c, Pc, and **QEA** in nonexpanded animals. The changes in .hivin..pi.c, Pc, and **QEA** induced in the euvolemic non-diuretic rats were the same as those in the saline-expanded diuretic rats. Under euvolemic experimental conditions, UNa V and APR do not correlate with intracapillary pressures or flow rates in the renal cortex. The only difference between the nondiuretic and diuretic rats was a rise in Pi in the latter group.

1984

1/AB/11 (Item 1 from file: 10)
DIALOG(R)File 10:AGRICOLA

3830599 22050289 Holding Library: AGL
Quantitative expression analysis of genes regulated by both obesity and leptin reveals a regulatory loop between leptin and pituitary-derived ACTH
Renz, M. Tomlinson, E.; Hultgren, B.; Levin, N.; Gu, Q.M.; Shimkets, R.A.; Lewin, D.A.; Stewart, T.A.
Genentech, Inc., San Francisco, CA.

Bethesda, Md. : American Society for Biochemistry and Molecular Biology. The Journal of biological chemistry. Apr 7, 2000. v. 275 (14) p. 10429-10436.
ISSN: 0021-9258 CODEN: JBCHA3
DNAL CALL NO: 381 J824
Language: English

1/AB/12 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09952105 Genuine Article#: 467AG Number of References: 23
Title: Comparison of **RNA** and cDNA transfection methods for rescue of infectious bursal disease virus (ABSTRACT AVAILABLE)
Author(s): Boot HJ (REPRINT) ; Dokic K; Peeters BP
Corporate Source: ID Lelystad,Inst Anim Sci & Hlth, Dept Avian Virol,POB 65/NL-8200 AB Lelystad//Netherlands/ (REPRINT); ID Lelystad,Inst Anim Sci & Hlth, Dept Avian Virol,NL-8200 AB Lelystad//Netherlands/
Journal: JOURNAL OF VIROLOGICAL METHODS, 2001, V97, N1-2 (SEP), P67-76
ISSN: 0166-0934 Publication date: 20010900
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
Language: English Document Type: ARTICLE
Abstract: Specific alterations in the genetic material of **RNA** viruses rely on a technique known as reverse genetics. Transfection of cells with the altered generic material is a critical step of this procedure. In this report we have compared **RNA** and cDNA transfection methods for the efficiency of transient protein expression and rescue of (recombinant) infectious bursal disease virus (IBDV). **Quantitative expression analysis** of the secreted alkaline phosphatase reporter protein, and qualitative expression levels of an IBDV protein showed both that cDNA transfection results in a much higher level of protein expression than **RNA** transfection. Because the rescue of a crippled variant of IBDV was achieved consistently using the cDNA transfection method, but failed when we used the **RNA** transfection method, we favor the cDNA transfection method for the rescue of (recombinant) IBDV from cloned cDNA.

1/AB/13 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09704629 Genuine Article#: 438GQ Number of References: 31
Title: Comprehensive genome sequence analysis of a breast cancer amplicon (ABSTRACT AVAILABLE)
Author(s): Collins C (REPRINT) ; Volik S; Kowbel D; Ginzinger D; Ylstra B; Cloutier T; Hawkins T; Predki P; Martin C; Wernick M; Kuo WL; Alberts A ; Gray JW
Corporate Source: Univ Calif San Francisco,Ctr Canc,San Francisco//CA/94143 (REPRINT); Univ Calif San Francisco,Ctr Canc,San Francisco//CA/94143; Lawrence Berkeley Lab,Berkeley//CA/94143; Joint Genome Inst,Dept Energy,Walnut Creek//CA/94958; Novartis Agr Discovery Inst,San

Diego//CA/92121; Van Andel Inst,Grand Rapids//MI/49503
Journal: GENOME RESEARCH, 2001, V11, N6 (JUN), P1034-1042
ISSN: 1088-9051 Publication date: 20010600
Publisher: COLD SPRING HARBOR LAB PRESS, 1 BUNGTOWN RD, PLAINVIEW, NY 11724 USA
Language: English Document Type: ARTICLE
Abstract: Gene amplification occurs in most solid tumors and is associated with poor prognosis. Amplification of 20q13.2 is common to several tumor types including breast cancer. The 1 Mb of sequence spanning the 20q13.2 breast cancer amplicon is one of the most exhaustively studied segments of the human genome. These studies have included amplicon mapping by comparative genomic hybridization (CGH), fluorescent in-situ hybridization (FISH), array-CGH, quantitative microsatellite analysis (QUMA), and functional genomic studies. Together these studies revealed a complex amplicon structure suggesting the presence of at least two driver genes in some tumors. One of these, ZNF217, is capable of immortalizing human mammary epithelial cells [HMEC) when overexpressed. In addition, we now report the sequencing of this region in human and mouse, and on quantitative expression studies in tumors. Amplicon localization now is straightforward and the availability of human and mouse genomic sequence facilitates their functional analysis. However, comprehensive annotation of megabase-scale regions requires integration of vast amounts of information. We present a system for integrative analysis and demonstrate its utility on 1.2 Mb of sequence spanning the 20q13.2 breast cancer amplicon and 865 kb of syntenic: murine sequence. We integrate tumor genome copy number measurements with exhaustive genome landscape mapping, showing that amplicon boundaries are associated with maxima in repetitive element density and a region of evolutionary instability. This integration of comprehensive sequence annotation, **quantitative expression analysis**, and tumor amplicon boundaries provide evidence for an additional driver gene prefoldin 4 (PFDN4), coregulated genes, conserved noncoding regions, and associate repetitive elements with regions of genomic instability at this locus.

1/AB/14 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09542072 Genuine Article#: 416ZL Number of References: 49
Title: Open systems: panoramic views of gene expression (ABSTRACT AVAILABLE)
Author(s): Green CD; Simons JF; Taillon BE; Lewin DA (REPRINT)
Corporate Source: CuraGen Corp,Dept Gene Discovery,555 Long Wharf Dr/New Haven//CT/06511 (REPRINT); CuraGen Corp,Dept Gene Discovery,New Haven//CT/06511; CuraGen Corp,Dept Engn & Technol Dev,New Haven//CT/06511
Journal: JOURNAL OF IMMUNOLOGICAL METHODS, 2001, V250, N1-2 (APR 1), P67-79
ISSN: 0022-1759 Publication date: 20010401
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
Language: English Document Type: ARTICLE
Abstract: Since their development in the early 1990s, differential gene expression (DGE) technologies have been applied to a multitude of

biological challenges, both for the purpose of basic biological research and as a valuable tool for the discovery and development of pharmaceuticals. In this review we survey a class of DGE technologies collectively referred to as 'open' architecture systems. These technologies are distinct from the 'closed' DGE technologies (quantitative PCR, chip technologies), in that no pre-existing biological or sequence information is necessary and they are applicable to any species. Examples of open systems include GeneCalling (R):, SAGE, TOGA, READS (TM) and their progenitor DGE technologies, differential display and cDNA representational difference analysis. We review these technologies and summarize a specific application using GeneCalling for novel gene discovery. Additionally, the significance of data management and experimental design in this new age of expression analysis is discussed.

1/AB/15 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09248342 Genuine Article#: 383WR Number of References: 19
Title: **Quantitative expression analysis** of the cellular specificity of HECT-domain ubiquitin E3 ligases (ABSTRACT AVAILABLE)
Author(s): Scarafia LE; Winter A; Swinney DC (REPRINT)
Corporate Source: Roche Biosci,Inflammatory Dis Unit,M-S S3-1,3401 Hillview Ave/Palo Alto//CA/94304 (REPRINT); Roche Biosci,Inflammatory Dis Unit,Palo Alto//CA/94304
Journal: PHYSIOLOGICAL GENOMICS, 2000, V4, N2 (DEC 18), P147-153
ISSN: 1094-8341 Publication date: 20001218
Publisher: AMER PHYSIOLOGICAL SOC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA
Language: English Document Type: ARTICLE
Abstract: We evaluated the expression of 28 gene sequences with homology to the carboxy terminal of HECT E3 ubiquitin ligases in nine human cell lines using RT-PCR, to determine whether gene expression could be associated with cell-specific functions (HECT is "homologous to E6AP C-terminus"). In general, HECT-domain E3 ligases are constitutively expressed at low levels with a broad range between cell types. hecth3, 21, and 23 had higher levels in three leukocytic lines (Jurkat, MM6, THP1); hecth11 was more abundant in HepG2 and A495; and hecth15 and hecth12 were differentially expressed in lung fibroblasts derived from normal and severe emphysema patients (CCD16 and CCD29, respectively). Absolute quantitation showed that most HECT E3s have about 20-100 copies of mRNA per Jurkat cell. By comparison, UBCH7 (an ubiquitin-conjugating E2) is 10-fold more abundant in Jurkat cells and 30-fold more abundant than E2 UBCH5A. We interpret the broad range of transcript levels to be consistent with the hypothesis that the concentrations of E3 are important for ubiquitination selectivity, leading us to conclude that substrate activation is necessary but not sufficient for selectivity.

1/AB/16 (Item 5 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

08569416 Genuine Article#: 302BT Number of References: 58
Title: **Quantitative expression analysis** of genes regulated by both obesity and leptin reveals a regulatory loop between leptin and pituitary-derived ACTH (ABSTRACT AVAILABLE)
Author(s): Renz M; Tomlinson E; Hultgren B; Levin N; Gu QM; Shimkets RA; Lewin DA; Stewart TA (REPRINT)
Corporate Source: GENENTECH INC,DEPT ENDOCRINE RES, 1 DNA WAY/S SAN FRANCISCO//CA/94080 (REPRINT); GENENTECH INC,DEPT ENDOCRINE RES/S SAN FRANCISCO//CA/94080; CURAGEN CORP,GENE DISCOVERY/NEW HAVEN//CT/06511
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 2000, V275, N14 (APR 7), P 10429-10436
ISSN: 0021-9258 Publication date: 20000407
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
Language: English Document Type: ARTICLE
Abstract: Absence of the hormone leptin leads to dramatic increases in appetite, food intake, and adiposity, The primary site of action, at least with respect to appetite, is the hypothalamus. Leptin also has significant effects on the function(s) of peripheral organs involved in maintaining body composition. Some of these effects are mediated through direct interaction of leptin with its receptor on the target tissue, and some effects are indirectly mediated through secondary hormonal and neural pathways. Few of the genes that are responsible for regulating body composition and the peripheral effects of leptin are known. We have used a new gene profiling technology to characterize gene expression changes that occur in the pituitary, hypothalamus, fat, muscle, and liver in response to both obesity and treatment with exogenous leptin, These differences were then overlaid to allow the identification of genes that are regulated by obesity and at least partially normalized by leptin treatment. By using this process we have identified five genes (POMC, PC2, prolactin, HSGP25L2G, and one novel) that are both abnormally expressed in the pituitaries of obese mice and are sensitive to the effects of leptin, We also show that adrenocorticotropic hormone appears to be involved in a regulatory loop involving leptin.

1/AB/17 (Item 6 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

08460447 Genuine Article#: 288BQ Number of References: 41
Title: Methacarn fixation: A novel tool for analysis of gene expressions in paraffin-embedded tissue specimens (ABSTRACT AVAILABLE)
Author(s): Shibutani M (REPRINT) ; Uneyama C; Miyazaki K; Toyoda K; Hirose M
Corporate Source: NATL INST HLTH SCI,DIV PATHOL, SETAGAYA KU, 1-18-1 KAMIYOGA/TOKYO 1588501//JAPAN/ (REPRINT)
Journal: LABORATORY INVESTIGATION, 2000, V80, N2 (FEB), P199-208
ISSN: 0023-6837 Publication date: 20000200
Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA

19106-3621
Language: English Document Type: ARTICLE
Abstract: To establish a quantitative method for analysis of gene expressions in small areas of tissue after paraffin embedding, preliminary validation experiments with RT-PCR and Western blotting were performed using methacarn-fixed rodent tissues and a cultured PC12 cell line. A total **RNA** yield of 52 +/- 15 ng/mm(2), sufficient for a quantitative RT-PCR of many genes, could be extracted from a deparaffinized 10-mu m-thick rat-liver section by a simple, single-step extraction method. The low concentration of contaminating genomic **DNA** and the resolution of ribosomal RNAs in **RNA** gel proved the purity and integrity of the extracted **RNA** samples, allowing PCR amplification of a long mRNA sequence and mRNA species expressing low copy numbers. PCR amplification of mRNA-derived target gene fragments could be achieved by optimizing the amount of total **RNA** for reverse transcription and the number of subsequent PCR cycles for each gene. By this validation, organ- and sex-specific mRNA expression could be detected in methacarn-fixed paraffin-embedded tissues without additional DNase treatment of **RNA** samples. RT-PCR analysis could also be performed with total **RNA** extracted from deparaffinized tissue dissected with a laser capture microdissection system. In addition, extraction of protein yielded 4.9 +/- 2.1 mu g/mm(2) from a 10-mu m-thick rat-liver section, allowing a **quantitative expression analysis** of protein by Western blotting. Thus, in addition to its advantages for immunohistochemistry, methacarn-fixed paraffin-embedded tissue has benefits for analysis of both RNAs and proteins in the cells of histologically defined areas.

1/AB/18 (Item 7 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05743726 Genuine Article#: WU885 Number of References: 30
Title: Differential and constitutive expression of the DRB1 and DRA gene products controls the surface HLA-DR expression level in human eosinophilic leukaemia cell lines (ABSTRACT AVAILABLE)
Author(s): Nakatsuji T (REPRINT)
Corporate Source: TOKAI UNIV,GRAD SCH MARINE SCI & TECHNOL, DEPT MARINE BIOL SCI, 3-20-1 ORIDO/SHIMIZU/SHIZUOKA 424/JAPAN/ (REPRINT)
Journal: CELL STRUCTURE AND FUNCTION, 1997, V22, N1 (FEB), P15-20
ISSN: 0386-7196 Publication date: 19970200
Publisher: JAPAN SOC CELL BIOLOGY, SHIMOTACHIURI OGAWA-HIGASHI, KAMIKYOKU KYOTO 602, JAPAN
Language: English Document Type: ARTICLE
Abstract: Use of 2-D gel and imaging plate analysis enabled biosynthetically radiolabeled immunoprecipitates to be quantitated at the very low level of gene products during processing from RER inside cells to cell surface. We used this efficient and sensitive measurement to analyse expression of HLA-DR molecules in human eosinophilic leukaemia cell lines. We found that they synthesized a constitutive amount of DRA gene products and differential amounts of DRB1 gene products. Thus, the incompletely inducible expression of DRB1 gene products was responsible for the limited accumulation of normally

assembled molecules for cell surface expression and the lack of serological determination.

1/AB/19 (Item 1 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

01843545 2001205479
Comprehensive genome sequence analysis of a breast cancer amplicon
Collins C.; Volik S.; Kowbel D.; Ginzinger D.; Ylstra B.; Cloutier T.; Hawkins T.; Predki P.; Martin C.; Wernick M.; Kuo W.-L.; Alberts A.; Gray J.W.
ADDRESS: C. Collins, University of California, San Francisco Cancer Center, San Francisco, CA 94143-0808, United States
EMAIL: collins@cc.ucsf.edu
Journal: Genome Research, 11/6 (1034-1042), 2001, United States
CODEN: GEREF
ISSN: 1088-9051
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 31

Gene amplification occurs in most solid tumors and is associated with poor prognosis. Amplification of 20q13.2 is common to several tumor types including breast cancer. The 1 Mb of sequence spanning the 20q13.2 breast cancer amplicon is one of the most exhaustively studied segments of the human genome. These studies have included amplicon mapping by comparative genomic hybridization (CGH), fluorescent in-situ hybridization (FISH), array-CGH, quantitative microsatellite analysis (QUMA), and functional genomic studies. Together these studies revealed a complex amplicon structure suggesting the presence of at least two driver genes in some tumors. One of these, ZNF217, is capable of immortalizing human mammary epithelial cells (HMEC) when overexpressed. In addition, we now report the sequencing of this region in human and mouse, and on quantitative expression studies in tumors. Amplicon localization now is straightforward and the availability of human and mouse genomic sequence facilitates their functional analysis. However, comprehensive annotation of megabase-scale regions requires integration of vast amounts of information. We present a system for integrative analysis and demonstrate its utility on 1.2 Mb of sequence spanning the 20q13.2 breast cancer amplicon and 865 kb of syntenic murine sequence. We integrate tumor genome copy number measurements with exhaustive genome landscape mapping, showing that amplicon boundaries are associated with maxima in repetitive element density and a region of evolutionary instability. This integration of comprehensive sequence annotation, **quantitative expression analysis**, and tumor amplicon boundaries provide evidence for an additional driver gene prefoldin 4 (PFDN4), coregulated genes, conserved noncoding regions, and associate repetitive elements with regions of genomic instability at this locus.

1/AB/20 (Item 2 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

01814296 2001176230
Comparison of **RNA** and cDNA transfection methods for rescue of infectious bursal disease virus
Boot H.J.; Dokic K.; Peeters B.P.H.
ADDRESS: H.J. Boot, Department of Avian Virology, Institute for Animal Science/Health, PO Box 65, NL-8200 AB Lelystad, Netherlands
EMAIL: h.j.boot@id.wag-ur.nl
Journal: Journal of Virological Methods, 97/1-2 (67-76), 2001, Netherlands
CODEN: JVMED
ISSN: 0166-0934
PUBLISHER ITEM IDENTIFIER: S0166093401003408
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 23

Specific alterations in the genetic material of **RNA** viruses rely on a technique known as reverse genetics. Transfection of cells with the altered generic material is a critical step of this procedure. In this report we have compared **RNA** and cDNA transfection methods for the efficiency of transient protein expression and rescue of (recombinant) infectious bursal disease virus (IBDV). **Quantitative expression analysis** of the secreted alkaline phosphatase reporter protein, and qualitative expression levels of an IBDV protein showed both that cDNA transfection results in a much higher level of protein expression than **RNA** transfection. Because the rescue of a crippled variant of IBDV was achieved consistently using the cDNA transfection method, but failed when we used the **RNA** transfection method, we favor the cDNA transfection method for the rescue of (recombinant) IBDV from cloned cDNA. (c) 2001 Elsevier Science B.V. All rights reserved.

1/AB/21 (Item 3 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

01378138 2000053965
Methacarn fixation: A novel tool for analysis of gene expressions in paraffin-embedded tissue specimens
Shibutani M.; Uneyama C.; Miyazaki K.; Toyoda K.; Hirose M.
ADDRESS: Dr. M. Shibutani, Division of Pathology, Natl. Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
EMAIL: shibutan@nihs.go.jp
Journal: Laboratory Investigation, 80/2 (199-208), 2000, United States
CODEN: LAINA
ISSN: 0023-6837
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 41

To establish a quantitative method for analysis of gene expressions in small areas of tissue after paraffin embedding, preliminary validation experiments with RT-PCR and Western blotting were performed using

methacarn- fixed rodent tissues and a cultured PC12 cell line. A total **RNA** yield of 52 +/- 15 ng/mmsup 2, sufficient for a quantitative RT-PCR of many genes, could be extracted from a deparaffinized 10-mum-thick rat-liver section by a simple, single-step extraction method. The low concentration of contaminating genomic **DNA** and the resolution of ribosomal RNAs in **RNA** get proved the purity and integrity of the extracted **RNA** samples, allowing PCR amplification of a long mRNA sequence and mRNA species expressing low copy numbers. PCR amplification of mRNA-derived target gene fragments could be achieved by optimizing the amount of total **RNA** for reverse transcription and the number of subsequent PCR cycles for each gene. By this validation, organ- and sex-specific mRNA expression could be detected in methacarn-fixed paraffin-embedded tissues without additional DNase treatment of **RNA** samples. RT-PCR analysis could also be performed with total **RNA** extracted from deparaffinized tissue dissected with a laser capture microdissection system. In addition, extraction of protein yielded 4.9 +/- 2.1 mug/mmsup 2 from a 10-mum-thick rat-liver section, allowing a **quantitative expression analysis** of protein by Western blotting. Thus, in addition to its advantages for immunohistochemistry, methacarn-fixed paraffin-embedded tissue has benefits for analysis of both RNAs and proteins in the cells of histologically defined areas.

1/AB/22 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

11260741 EMBASE No: 2001275394
Comparison of **RNA** and cDNA transfection methods for rescue of infectious bursal disease virus
Boot H.J.; Dokic K.; Peeters B.P.H.
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Journal of Virological Methods (J. VIROL. METHODS) (Netherlands) 2001, 97/1-2 (67-76)
CODEN: JVMED ISSN: 0166-0934
PUBLISHER ITEM IDENTIFIER: S0166093401003408
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 23

Specific alterations in the genetic material of **RNA** viruses rely on a technique known as reverse genetics. Transfection of cells with the altered generic material is a critical step of this procedure. In this report we have compared **RNA** and cDNA transfection methods for the efficiency of transient protein expression and rescue of (recombinant) infectious bursal disease virus (IBDV). **Quantitative expression analysis** of the secreted alkaline phosphatase reporter protein, and qualitative expression levels of an IBDV protein showed both that cDNA transfection results in a much higher level of protein expression than **RNA** transfection. Because the rescue of a crippled variant of IBDV was achieved consistently using the cDNA transfection method, but failed when we used the **RNA** transfection method, we favor the cDNA

transfection method for the rescue of (recombinant) IBDV from cloned cDNA.

1/AB/23 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

11173049 EMBASE No: 2001182623
Isolation and characterization of a CaSUP2+-activated chloride channel from human corneal epithelium
Itoh R.; Kawamoto S.; Miyamoto Y.; Kinoshita S.; Okubo K.
K. Okubo, Inst. for Molecular/Cellular Biology, Osaka University, 1-3, Yamada-oka, Osaka 565-0871 Japan
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Current Eye Research (CURR. EYE RES.) (Netherlands) 2000, 21/6 (918-925)
CODEN: CEYRD ISSN: 0271-3683
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 39

Purpose. Transparency of the cornea is maintained through the activity of secretory mechanisms in the epithelium and endothelium, which offset the tendency of the stroma to imbibe fluid and swell. These secretory mechanisms establish osmotic gradients thereby providing the osmotic driving forces for coupled fluid transport from the stroma into both the tears and the anterior chamber. To further characterize the mechanism of epithelial Cl secretion, we cloned a cDNA encoding a CaSUP2+-dependent chloride channel, an abundant mRNA in human corneal epithelium. We investigated the abundance of all known human chloride channels in corneal epithelium to identify those responsible for regulating chloride conductance in this tissue. Methods. For the isolation of a full-length cDNA clone, a probe was selected from a set of expressed sequenced tag (EST) clones classified as unique to corneal epithelium (http://bodymap.ims.u-tokyo.ac.jp). The expression patterns of the corresponding gene encoding novel chloride channel gene in human cornea and other tissues were examined by reverse transcription-polymerase chain reaction (RT-PCR). Quantitative PCR was performed to clarify the expression level of the novel chloride channel gene in cornea relative to that in other human tissues. Results. We cloned a new CaSUP2+-activated chloride channel, CLCA2, from corneal epithelium. The full length cDNA clone encoded 943 amino acids with 62% identity to bovine CaSUP2+activated chloride channel. The CLCA2 gene mapped to human chromosome 1p32. **Quantitative expression analysis** by RT-PCR showed that it is the most abundant chloride channel in corneal epithelium. Conclusion. High and tissue specific expression of the CLCA2 gene in human corneal epithelium implies an important role in corneal transparency maintenance.

1/AB/24 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE

10616792 EMBASE No: 2000082078
Methacarn fixation: A novel tool for analysis of gene expressions in paraffin-embedded tissue specimens
Shibutani M.; Uneyama C.; Miyazaki K.; Toyoda K.; Hirose M.
Dr. M. Shibutani, Division of Pathology, Natl. Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501 Japan
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Laboratory Investigation (LAB. INVEST.) (United States) 2000, 80/2 (199-208)
CODEN: LAINA ISSN: 0023-6837
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 41

To establish a quantitative method for analysis of gene expressions in small areas of tissue after paraffin embedding, preliminary validation experiments with RT-PCR and Western blotting were performed using methacarn- fixed rodent tissues and a cultured PC12 cell line. A total **RNA** yield of 52 +/- 15 ng/mmsup 2, sufficient for a quantitative RT-PCR of many genes, could be extracted from a deparaffinized 10-mum-thick rat-liver section by a simple, single-step extraction method. The low concentration of contaminating genomic **DNA** and the resolution of ribosomal RNAs in **RNA** get proved the purity and integrity of the extracted **RNA** samples, allowing PCR amplification of a long mRNA sequence and mRNA species expressing low copy numbers. PCR amplification of mRNA-derived target gene fragments could be achieved by optimizing the amount of total **RNA** for reverse transcription and the number of subsequent PCR cycles for each gene. By this validation, organ- and sex-specific mRNA expression could be detected in methacarn-fixed paraffin-embedded tissues without additional DNase treatment of **RNA** samples. RT-PCR analysis could also be performed with total **RNA** extracted from deparaffinized tissue dissected with a laser capture microdissection system. In addition, extraction of protein yielded 4.9 +/- 2.1 mug/mmsup 2 from a 10-mum-thick rat-liver section, allowing a **quantitative expression analysis** of protein by Western blotting. Thus, in addition to its advantages for immunohistochemistry, methacarn-fixed paraffin-embedded tissue has benefits for analysis of both RNAs and proteins in the cells of histologically defined areas.

1/AB/25 (Item 4 from file: 73)
DIALOG(R)File 73:EMBASE

07475317 EMBASE No: 1998410120
Distinct molecular phenotypes in murine cardiac muscle development, growth, and hypertrophy
Schoenfeld J.R.; Vasser M.; Jhurani P.; Ng P.; Hunter J.J.; Ross J. Jr.; Chien K.R.; Lowe D.G.
Dr. D.G. Lowe, Cardiovascular Research, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080 United States
Journal of Molecular and Cellular Cardiology (J. MOL. CELL. CARDIOL.) (United Kingdom) 1998, 30/11 (2269-2280)
CODEN: JMCDA ISSN: 0022-2828

DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 43

The onset of cardiac hypertrophy is associated with characteristic changes in myocardial gene expression that are thought to recapitulate a developmental gene program. We report here the first gene expression profile of the murine myocardium, using a rapid method of **quantitative expression analysis** based on real-time analytical RT-PCR. This assay was used to measure expression levels of 29 genes in (1) late stage development as represented by day 1 neonatal ventricles, (2) normal cardiac growth in 3 and 18 month old mice, and (3) cardiac hypertrophy following pressure overload by aortic constriction. For males and females normal growth is not associated with differential expression although there is elevated expression of skeletal and smooth muscle actin mRNA's in males compared to females. Using normal adult ventricles as a reference, there are many qualitative and quantitative differences between the day 1 neonatal myocardium and experimental cardiac hypertrophy. These data suggest that the response to POL involves a subset of re-expressed developmental genes together with altered expression of genes not necessarily associated with cardiac development.

1/AB/26 (Item 1 from file: 76)
DIALOG(R)File 76:Life Sciences Collection

02624465 5155972
Comparison of **RNA** and cDNA transfection methods for rescue of infectious bursal disease virus
Boot, H.J.; Dokic, K.; Peeters, B.P.H.
Department of Avian Virology, Institute for Animal Science and Health, ID-Lelystad, PO Box 65, NL-8200 AB Lelystad The Netherlands
Journal of Virological Methods vol. 97, no. 1-2, pp. 67 - 76 (2001)
ISSN: 0166-0934
DOCUMENT TYPE: Journal article LANGUAGE: ENGLISH
SUBFILE: Virology & AIDS Abstracts; Microbiology Abstracts A: Industrial & Applied Microbiology

Specific alterations in the genetic material of **RNA** viruses rely on a technique known as reverse genetics. Transfection of cells with the altered generic material is a critical step of this procedure. In this report we have compared **RNA** and cDNA transfection methods for the efficiency of transient protein expression and rescue of (recombinant) infectious bursal disease virus (IBDV). **Quantitative expression analysis** of the secreted alkaline phosphatase reporter protein, and qualitative expression levels of an IBDV protein showed both that cDNA transfection results in a much higher level of protein expression than **RNA** transfection. Because the rescue of a crippled variant of IBDV was achieved consistently using the cDNA transfection method, but failed when we used the **RNA** transfection method, we favor the cDNA transfection method for the rescue of (recombinant) IBDV from cloned cDNA.

1/AB/27 (Item 2 from file: 76)
DIALOG(R)File 76:Life Sciences Collection

02132157 4039675
Advancing technologies in gene amplification
Vrana, K.E.
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TRENDS BIOTECHNOL. vol. 14, no. 11, pp. 413-415 (1996)
ISSN: 0167-7799
DOCUMENT TYPE: Journal article; Review article LANGUAGE: ENGLISH
SUBFILE: Medical and Pharmaceutical Biotechnology Abstracts

Shakespeare's classic line from the end of the sixteenth century is particularly appropriate when considering the current status of gene amplification technology. Presentations at a recent meeting on advances in this field indicated that there is a variety of new amplification techniques to supplement traditional PCR for the detection of rare **nucleic acid** sequences. Amplification technologies now include **Nucleic Acid**-Based Sequence Amplification (NASBA), Transcription-Mediated Amplification (TMA), Strand Displacement Amplification (SDA), aRNA amplification, branched **DNA** (bDNA) amplification, hybrid capture, Multiple Allele-Specific Diagnostic Assay (MASDA), **Quantitative Expression Analysis** (**QEA**), both competitive and noncompetitive reverse-transcriptase PCR (RT-PCR), and in situ PCR and this list considers only the experimental paradigms. Experimental output (signal detection) also encompasses a variety of modalities from traditional super(32P) incorporation (assessed by phosphor-imaging, liquid scintillation spectrometry and autoradiography), to HPLC coupled with uv spectroscopy, to luminometry, and ultimately to real-time fluorescence monitoring of the amplification process. Details of most of the main systems and technologies were presented.

1/AB/28 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

14611313 PASCAL No.: 00-0280558
Methacarn fixation : A novel tool for analysis of gene expressions in paraffin-embedded tissue specimens
SHIBUTANI M; UNEYAMA C; MIYAZAKI K; TOYODA K; HIROSE M
Division of Pathology, National Institute of Health Sciences, Kamiyoga, Setagaya-ku, Tokyo, Japan
Journal: Laboratory investigation, 2000, 80 (2) 199-208
Language: English
To establish a quantitative method for analysis of gene expressions in small areas of tissue after paraffin embedding, preliminary validation experiments with RT-PCR and Western blotting were performed using methacarn-fixed rodent tissues and a cultured PC12 cell line. A total **RNA** yield of 52 +- 15 ng/mm SUP 2 , sufficient for a quantitative RT-PCR of many genes, could be extracted from a deparaffinized 10- mu m-thick rat-liver section by a simple, single-step extraction method. The low concentration of contaminating genomic **DNA** and the resolution of

ribosomal RNAs in **RNA** gel proved the purity and integrity of the extracted **RNA** samples, allowing PCR amplification of a long mRNA sequence and mRNA species expressing low copy numbers. PCR amplification of mRNA-derived target gene fragments could be achieved by optimizing the amount of total **RNA** for reverse transcription and the number of subsequent PCR cycles for each gene. By this validation, organ- and sex-specific mRNA expression could be detected in methacarn-fixed paraffin-embedded tissues without additional DNase treatment of **RNA** samples. RT-PCR analysis could also be performed with total ~~RNA~~ extracted ~~from deparaffinized~~ tissue dissected with a laser capture microdissection system. In addition, extraction of protein yielded 4.9 +- 2.1 mu g/mm SUP 2 from a 10- mu m-thick rat-liver section, allowing a **quantitative expression analysis** of protein by Western blotting. Thus, in addition to its advantages for immunohistochemistry, methacarn-fixed paraffin-embedded tissue has benefits for analysis of both RNAs and proteins in the cells of histologically defined areas.